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Article

RETRACTED: Health Risk Assessment of Pesticide Residues via Dietary Intake of Market Vegetables from Dhaka, Bangladesh

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Abstract: The present study was designed to assess the health risk of pesticide residues via dietary intake of vegetables collected from four top agro-based markets of Dhaka, Bangladesh. High performance liquid chromatography with a photo diode array detector (HPLC-PDA) was used to determine six organophosphorus (chlorpyrifos, fenitrothion, parathion, ethion, acephate, fenthion), two carbamate (carbaryl and carbofuran) and one pyrethroid (cypermethrin) pesticide residues in twelve samples of three common vegetables (tomato, lady's finger and brinjal). Pesticide residues ranged from below detectable limit (<0.01) to 0.36 mg/kg. Acephate, chlorpyrifos, ethion, carbaryl and cypermethrin were

detected in only one sample, while co-occurrence occurred twice for fenitrothion and parathion. Apart from chlorpyrifos in tomato and cypermethrin in brinjal, all pesticide residues exceeded the maximum residue limit (MRL). Hazard risk index (HRI) for ethion (10.12) and carbaryl (1.09) was found in lady's finger and tomato, respectively. Rest of the pesticide residues were classified as not a health risk.
A continuous monitoring and strict regulation should be enforced regarding control of pesticide residues in vegetables and other food commodities.

Keywords: pesticide residues; vegetables; health risk; health hazard; food safety; HRI; HPLC-PDA

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1. Introduction

Farmers around the world use different types of pesticides including organochlorines, organophosphorus, carbamate and pyrethroid insecticides, fungicides and herbicides against the possibility of a devastating crop loss from pests and diseases, as well as to increase agricultural productivity to provide adequate food supply for the increasing world population. Over the past couple of decades a rapid increase in the quantity and use of pesticides in the agricultural sector has been observed and this growth trend is expected to continue for the next decades due to several socio-economic and technological developments [1,2]. However, pesticide use has also been associated with several concerns, including the potential risks to human health from both occupational and non-occupational exposures, the death of farm animals and alteration of the local environment [3]. Many of these compounds can cause moderate to severe respiratory and neurological damage or act as genotoxic, carcinogenic and mutagenic agents, endocrine disruption, *etc.*, through routes that include consumption of dietary residues [4–6]. Very common effects of pesticide residues in human body include nausea, vomiting, blurred vision, coma, difficulty in breathing, deficit hyperactivity disorder, disorder in fetuses and children, *etc.* [7–9]. Many pesticides and their residues are also known to be contributory factors in several diseases such as cancer, heart diseases, Alzeheimer's and Parkinsonism [10]. The WHO [11] estimates an annual three million cases of acute and severe pesticide poisoning worldwide with some 220,000 deaths. The majority of these cases of poisoning and deaths occur in developing countries, although far greater quantities of pesticides are used in the developed countries [12].

Numerous indices can be used to predict pesticide residues intake. The maximum residue limit (MRL) is one such index which represents the maximum concentration of a pesticide residue (mg/kg) that the Codex Alimentarius Commission recommends be legally permitted in food commodities and animal feeds [13]. The acceptable daily intake (ADI) which is the estimated amount of a substance in food (expressed on a body weight basis) that can be ingested daily over a lifetime without appreciable health risk to the consumer. The estimated daily intake (EDI) of a pesticide residue in a given food is obtained by multiplying the residue level in the food by the amount of that food consumed. EDI of pesticide residues should be less than its established ADI [13–15]. The hazard risk index (HRI) is applied to assess the potential health risk from consumption of pesticide residues containing foodstuff.

Bangladesh is an agro-dominant country where about 62% of the population is involved directly or indirectly in the agricultural sector. The most alarming concern is indiscriminate use of pesticides due to their easy availability, relatively cheap cost and ease of application [16,17]. Actually, pesticide residues in food and crops are direct results of application of pesticides to agricultural field and to a lesser extent from pesticide residues contaminating the soil [18]. Moreover, sometimes few farmers do not wait long enough for the residues to wash off after spraying before harvesting because of their high demand for farm products and low perception of the toxic effects of pesticide residues in food [19]. Thus, increased use of pesticides in agriculture has resulted in the occurrence of residues in food commodities [15] that has always been a matter of serious concern especially when these commodities are consumed fresh [20]. Nevertheless, to the best of our knowledge, no investigations of regular surveys, monitoring and assessment have been reported on the concentration of pesticides residues in vegetables at the market level and related health risk assessment in Dhaka, the capital city of Bangladesh. In the present study health risk of a total of six organophosphorus (chlorpyrifos, fenitrothion, parathion, ethion, acephate, fenthion), two carbamate (carbaryl and carbofuran) and one pyrethroid (cypermethrin) pesticide residues was assessed via dietary intake of three common vegetables (tomato, lady's finger and brinjal) collected from some top agro-based markets of Dhaka. High performance liquid chromatography with photo diode array detector (HPLC-PDA) was used to determine the concentration of pesticide residues in vegetables and then HRI was calculated from the obtained results and other statistics.

2. Experimental Section

2.1. Sample Collection and Preparation

Twelve samples of three vegetables viz., brinjal, lady's finger and tomato were collected during winter season at random basis from four (Hatirpool, Raja Bazar, Karwon Bazar and Mahammudpur) vegetable markets of Dhaka City (Figure 1). These are top agro-based markets of Dhaka from where most of the vegetables are supplied to the city dwellers. The details of different vegetable samples during the experiment are given in Table 1. Sampling was conducted according to the international standard guideline [21]. Samples were taken among commodities considering high consumption rate and relatively cheap to buy. Four different batches of samples were taken for analysis. The sample size was at least 1 kg. Each sample was kept in a separate sterile polyethene bag, sealed, labelled with unique sample identity, placed in ice chest box and transported to laboratory. Samples were stored at 4 °C until analysis was performed within 24 h. In the laboratory, samples were chopped and ground in an electric blender to obtain a homogenous composite. Then 50 g homogenized samples from mother vegetable were taken for further analysis.

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Figure 1. Map of Dhaka city showing the sampling sites.

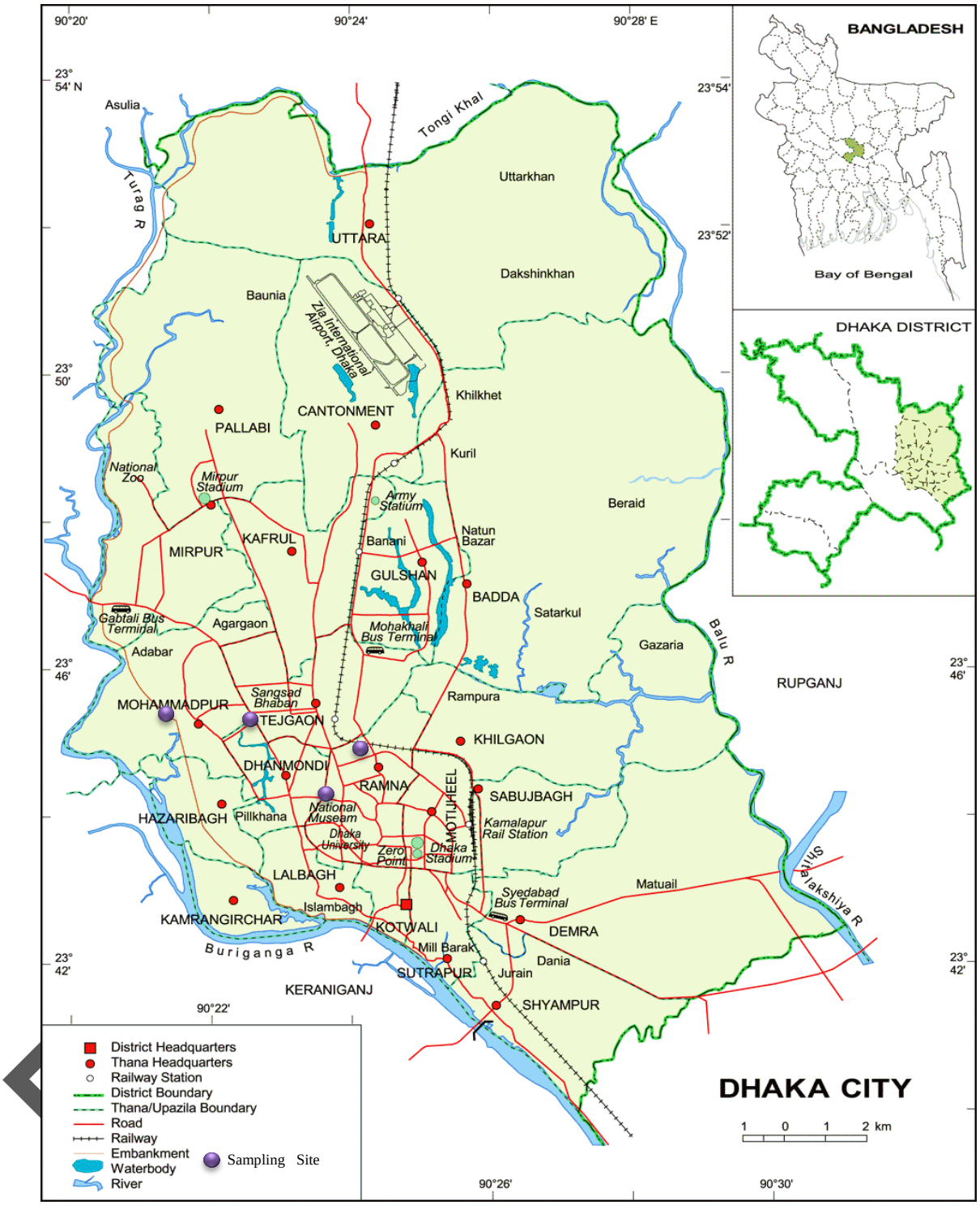

Table 1. Vegetable samples used for the present research.

Common Name	Scientific Name	Family	Edible Part
Tomato	*Lycopersicon esculentum* L.	Solanaceae	Fruit
Lady's Finger	*Abelmoschus esculentus* L.	Malvaceae	Fruit
Brinjal	*Solanum melongena* L.	Solanaceae	Fruit

2.2. Chemicals

Analytical grade acetone (BDH, Poole, England) and *n*-hexane (Merck, Germany) and HPLC grade acetonitrile (Scharlau, Barcelona, Spain) were used as main solvents. Anhydrous Sodium Sulfate (Na_2SO_4) (BDH, Poole, England), Florisil (Magnesium Silicate) (Sigma, St. Louis, MO, USA) and Dichloromethane (BDH, Poole, England) were also used while DD *n*-hexane was prepared in the laboratory. Acephate, chlorpyrifos, fenthion, fenitrothion, parathion, ethion, carbaryl, carbofuran and cypermethrin standards were of reference grade and purchased from Dr. Ehrenstorfer GmbH, D-86199 Augsburg, Germany.

2.3. Extraction

Fifty grams homogenized sample was extracted with 100 mL solvent mixture (hexane:dichloromethane = 9:1) in the presence of 20 g sodium hydrogen carbonate using electric blenders at about 20–25 °C. Sixty grams sodium sulphate was added to remove remaining water. The slurry was well mixed. The sample solvent mixture was kept in a fume hood for about 15–30 min to let the solvent separate from the solid material. The separated solvent was transferred to round bottom flask and evaporated in a rotary evaporator (Rotavapor-215, Buchi, Flawil, Switzerland) at 45 °C under mild pressure and finally transferred to a vial, and the evaporation was continued with gentle nitrogen stream to nearly dryness. The extract was dissolved in hexane and made to a particular volume (5 mL).

2.4. Clean Up

The samples were cleaned up using the procedure described in elsewhere [22,23]. Florisil column chromatography was used to clean up the extract. The florisil (mesh size 60−100) was activated at 200 °C for 6 h and deactivated with 2% distilled water. The top 1.5 cm of the florisil column was packed with anhydrous sodium sulphate. Elution was done with a solvent mixture of double distilled hexane (65%) and dichloromethane (35%) at 5 mL/min. The elute was concentrated in a rotary vacuum evaporator and transferred to a vial. Solvents were completely removed under mild fresh nitrogen flow. The evaporated sample was dissolved in acetonitrile and made to a particular volume (5 mL) for analysis by HPLC.

2.5. HPLC Analysis

Following the sample clean up, aliquots of the final volume was quantified using a HPLC (Shimadzu, Kyoto, Japan) LC-10 ADvp, equipped with an SPD-M 10 Avp attached to a PDA (Shimadzu SPD-M 10 Avp, 200–800 nm). The analytical column was a C18 Reverse Phase Alltech (250 × 4.6 mm, 5 μm) that was maintained at 30 °C in a column oven. The mobile phase, a combination of 70% ACN and 30% water, was filtered using a cellulose filter of 0.45 μm before each use. The flow rate was 1.0 mL/min and all solvents used were of HPLC grade. Prior to HPLC analysis, the samples were passed through 0.45 μm of nylon (Alltech Associates, IL, USA) syringe filters. Twenty microliters samples were manually injected each time. The identification of the suspected pesticide was performed, relative to the retention time of the pure analytical standard. Quantification

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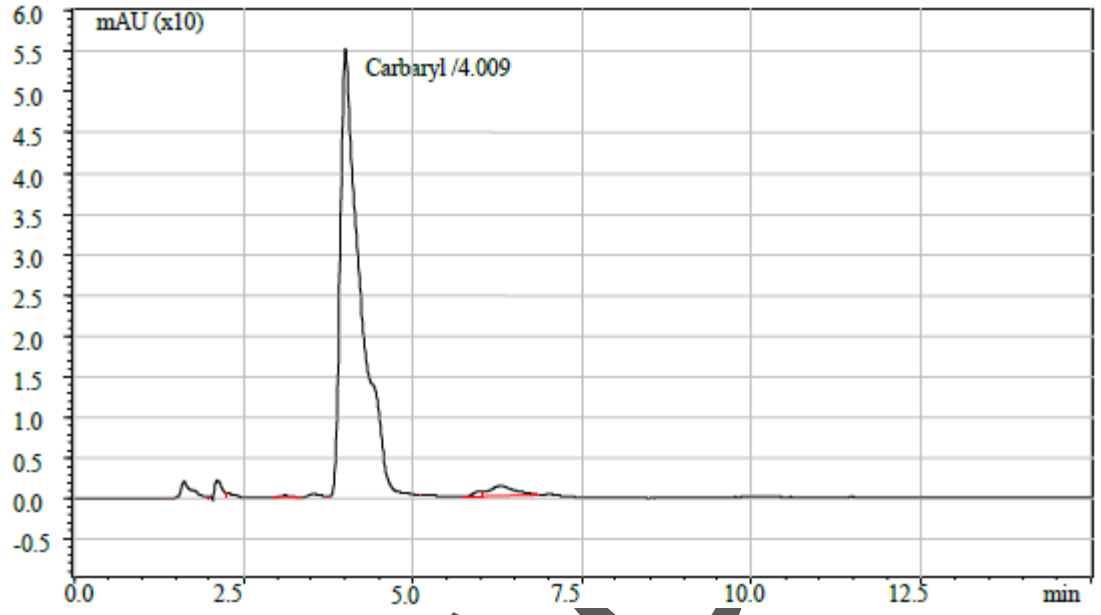
was performed based on the method described elsewhere [16,17]. A typical chromatogram from the analysis is shown in Figure 2.

Figure 2. Typical chromatogram of carbaryl standard injected at 50 mg/kg (retention time 4.009 min).



2.6. Recovery

The mean percentage recovery of pesticide residues were calculated using Equation (1):

$$\text{Percentage recovery} = (C_E/C_M) \times 100 \quad (1)$$

where C_E is the experimental concentration determined from the calibration curve and C_M is the spiked concentration.

2.7. Quantification Procedures

Tentative identification of the suspected pesticides was carried out in relation to the Retention Time (RT) of the pure analytical standards. For this purpose, equal volumes of differently concentrated standard solutions of each pesticide were injected into HPLC to prepare calibration. The retention featured ±0.05% difference is acceptable. To determine the residual levels of pesticides, Equation (2) was used:

$$R' = H_A V_{END} W_{ST}/H_{ST} V_i G_S \quad (2)$$

where R' is pesticide residue in vegetable samples (mg/kg), G_s is sample weight (kg), V_{END} is terminal volume of the sample solution (mL), V_i is portion of volume (μL), V_{END} is injected into HPLC column (μL), W_{ST} is amount of standard pesticides injected with standard solvent (μg), H_A is peak area obtained from V_i (mm^2) and H_{ST} is peak area obtained from W_{ST} (mm^2). The calibration curves for acephate, chlorpyrifos, fenthion, fenitrothion, parathion, ethion, carbaryl, carbofuran and cypermethrin were prepared at 50 mg/kg.

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2.8. Hazard Risk Index (HRI) Analysis

From a potential health perspective, it is certainly important to compare exposure estimates to established toxicological criteria such as EDI. Actually EDI is a realistic estimation of pesticides residues exposure that was calculated in the agreement with the international guidelines [24,25]. EDI of pesticide residues for each combination of pesticide and commodity was calculated by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (kg/day) and dividing by a body weight of 60 kg for an adult people. The average daily vegetable intake for adult was considered to be 0.345 kg/person/day [26]. Then HRI of the residues was computed using the results and other statistics followed by Equation (3), modified after EFSA [27]:

$$HRI = EDI/ADI \quad (3)$$

where EDI is estimated daily intake, ADI is acceptable daily intake. HRI value more than 1 is considered as not safe for human health [15].

3. Results and Discussion

3.1. Level of Pesticide Residues in Vegetables

The level of pesticide residues found in the analyzed samples during the present study is outlined in Table 2.

Table 2. Mean value (mg/kg) of triplicates of pesticide residues in market vegetable samples.

Sample ID	Location	Acephate	Chlorpyrifos	Fenthion	Fenitrothion	Parathion	Ethion	Carbaryl	Carbofuran	Cypermethrin
BS-1	Hatirpool	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
BS-2	Raja Bazar	0.27	BDL	BDL	0.29	0.32	BDL	BDL	BDL	BDL
BS-3	Karwon Bazar	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.36
BS-4	Mahammudpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
TS-1	Hatirpool	BDL	BDL	BDL	BDL	BDL	BBL	BDL	BDL	BDL
TS-2	Raja Bazar	BDL	0.18	BDL	BDL	BDL	BDL	BDL	BDL	BDL
TS-3	Karwon Bazar	BDL	BDL	BDL	BDL	0.23	BDL	1.52	BDL	BDL
TS-4	Mahammudpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
LS-1	Hatirpool	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
LS-2	Raja Bazar	BDL	BDL	BDL	0.13	BDL	BDL	BDL	BDL	BDL
LS-3	Karwon Bazar	BDL	BDL	BDL	BDL	BDL	1.76	BDL	BDL	BDL
LS-4	Mahammudpur	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BS: Brinjal sample; TS: Tomato sample; LS: Lady's finger sample; BDL: Below detection limit (0.01 mg/kg).

Among the studied pesticides fenthion and carbofuran were absent in the samples. Pesticide residues ranged from BDL (<0.01) to 0.36 mg/kg. Acephate, chlorpyrifos, ethion, carbaryl and cypermethrin were detected in only one sample each, while co-occurrence followed twice in fenitrothion and parathion. Total four, three and two pesticides residues were found in brinjal, tomato and lady's finger sample, respectively. The present research found much higher fenitrothion residue in brinjal (eggplant) and lady's finger compared to other study [28]. Ethion, acephate, parathion and chlorpyrifos concentration was also higher than other studies [28–30]. Acephate was reported as

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0.053 mg/kg in lady's finger by Sinha *et al.* [28]. Chen *et al.* [30] have found the maximum concentration of acephate and fenitrothion as 4.082 and 0.651 mg/kg, respectively in different vegetables from Xiamen city, China. Carbaryl residue was detected in tomato sample in the present work as 1.52 mg/kg. Parent pesticides may be converted to their metabolite and hence, might remain below detection limit, though some previous studies have recorded carbamate residues in different vegetables in different region of the world [31–34]. Osman *et al.* [32] recorded the mean concentration of carbaryl residue as 0.10 mg/kg in tomato and 0.09 mg/kg in brinjal in Al-Qassim region of Saudi Arabia. One brinjal sample was found contaminated with cypermethrin and the concentration of pesticide residue found in the present study was higher than those found in other studies [30,35]. There may be several reasons for the occurrence of pesticide residues in vegetables. The most common reason was using different pesticides in agricultural land to kill insects to ensure proper growth of vegetable. Moreover, the majority of farmers do not have enough perception and knowledge about the nature of chemical pesticide and its effect on health by consuming pesticide residues [16,17]. Bio-accumulation and bio-magnification in food chain may also take place that ultimately leads to severe health problems.

Pesticide residues of the present study were compared with MRL (Table 3) established by European Union [36] and Codex Alimentarius Commission [13]. It was found that detected pesticide residues were higher than MRLs value in all vegetable samples except chlorpyrifos and cypermethrin in tomato and brinjal, respectively. However, the persistent nature of the pesticides is of great concern due to their bio-accumulative behaviour and toxic biological effects on human [37]. Continuous monitoring of residual pesticides level in different food materials from different areas is obvious to understand the trend of contamination.

Table 3. Maximum residue limit (MRL) (mg/kg) of identified pesticide residues in several vegetables.

Pesticide	Brinjal		Lady's Finger		Tomato	
	MRL [36]	MRL [13]	MRL [36]	MRL [13]	MRL [36]	MRL [13]
Acephate	0.02	NA	0.02	NA	0.02	1
Chlorpyrifos	0.5	0.5	0.5	NA	0.5	0.5
Fenitrothion	0.01	NA	0.01	NA	0.01	NA
Parathion	0.05	NA	0.05	NA	0.05	NA
Ethion	0.01	NA	0.01	NA	0.01	NA
Carbaryl	0.05	0.5	0.05	NA	0.5	5
Cypermethrin	0.5	0.03	0.5	0.5	0.5	0.5

NA: Not available for commodities analyzed.

3.2. Daily Intake and Health Risk Assessment

ADI, EDI and HRI of pesticide residues are given in Table 4. Health indices of acephate, fenitrothion, parathion and cypermethrin in brinjal were 0.54, 0.84, 0.37 and 0.047 respectively. The highest health indices were found for ethion (10.12) and carbaryl (1.09) in lady's finger and tomato respectively. Therefore the main health risk may be posed by ethion and carbaryl, while the remaining pesticide residues present no risk. It is noteworthy that dietary pesticide intakes estimated in this study considered

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only exposures from vegetables and did not include other food products including fruits, grains, dairy, fish, meats, *etc.* Therefore, estimates are not considered as total dietary exposure to the pesticides, nor do we consider drinking water, residential or occupational exposures. So, it is an underestimation of the total exposure of pesticides studied. Moreover, not all registered pesticides and all vegetable usually consumed were measured in this study. At the same time, processing factors were ignored, whereas fruits and vegetables are often peeled, cooked or boiled before consumption, resulting in an overestimation of the actual exposure to pesticide residues. Furthermore, the effect of pesticides on more vulnerable groups such as children and pregnant women could all affect these calculations [30]. At the same time, in many backgrounds no detectable amount of pesticide residues is found but this does not necessarily mean that the content is truly zero. The content may just be too low for detection with the currently available methods and technology [30,38].

Table 4. Health risk assessment based on acceptable daily intake (ADI) of pesticide residues in vegetables.

Pesticide	ADI [39]	Brinjal			Lady's Finger			Tomato		
	(mg/kg/day)	EDI (mg/kg/day)	HRI	Health Risk	EDI (mg/kg/day)	HRI	Health Risk	EDI (mg/kg/day)	HRI	Health Risk
Acephate	0.003	0.00155	0.52	No	-	-	-	-	-	-
Chlorpyrifos	0.003	-	-	-	-	-	-	0.00104	0.35	No
Fenitrothrion	0.002	0.00167	0.84	No	0.00075	0.38	No	-	-	-
Parathion	0.005	0.00184	0.37	No	-	-	-	0.00132	0.26	No
Ethion	0.001	-	-	-	0.01012	10.1	Yes	-	-	-
Carbaryl	0.008	-	-	-	-	-	-	0.0087	1.09	Yes
Cypermethrin	0.05	0.00207	0.04	No	-	-	-	-	-	-

-: Denotes EDI not established for the particular pesticide in particular vegetable.

4. Conclusions

Long term accumulation of pesticide residues in the human body via dietary intake of vegetables and other food commodities is a severe problem, as indiscriminate amounts of such pesticides are used in many countries. Moreover, controlling the pesticide levels seems to be a substantial contemporary public health problem to guarantee food quality and to evaluate the health risk. The present research was aimed to evaluate the possible health risk of pesticide residues via dietary intake of vegetables in Dhaka city, Bangladesh. HPLC-PDA was applied to determine six organophosphorus, two carbamate and one pyrethroid pesticide residues in different vegetable samples. The maximum level of pesticide residues was 0.36 mg/kg. Only one sample contained acephate, chlorpyrifos, ethion, carbaryl and cypermethrin. HRI for ethion and carbaryl made the vegetable risky for human health while others were found not to be a health risk. As pesticide residues can bio-accumulate and bio-magnify several fold in a food chain over time, continuous and strict monitoring programs should be enforced to check and limit these residual levels in food items.

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Conflict of Interest

The authors declare no conflict of interest.

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